

**EFFECT OF WEED EXTRACTS AGAINST PULSE BEETLE,
Callosobruchus chinensis L. (COLEOPTERA:
BRUCHIDAE) OF MUNG BEAN**

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Abstract

The n-hexane extracts of the weeds 'bhatpata' *Clerodendrum viscosum*, 'kashiature' *Cassia tora*, 'dhakishak' *Dryopteris filix-max*, 'bonmorich' *Croton bonpalandianum* and 'ghagra' *Xanthium strumarium* were used to evaluate their effectiveness for suppressing pulse beetle, *Callosobruchus chinensis* reared on mung bean *Vigna radiata* grains. The investigations were done with 1, 2 and 4% n-hexane extracts of the weeds and an untreated control. The weed extracts exhibited considerable effectiveness which varied with weed species, concentrations and exposure durations. The higher concentrations showed the higher rate of insect mortality, fecundity, adult emergence inhibition, and grain protection. The LC₅₀ values of the extracts ranged from 5.3 to 7.8, 4.7 to 6.5 and 4.1 to 6.0 g/100 ml at 24, 48 and 72 hours after treatment, respectively. The fecundity inhibition varied from 31.7 to 78.7%, adult emergence inhibition from 33.8 to 81.1%, and grain damage inhibition from 10.3 to 60.1% when 'bhatpata' with concentration of 1 g/100 ml and 'ghagra' with concentration of 4g/100 ml were applied, respectively. Among the tested weeds, ghagra (4g/100 ml) showed better efficacy against *C. chinensis* compared to other tested extracts and may be suggested to control pulse beetle and protection of mung bean grains.

Keywords: Adult emergence, bruchids, fecundity, grain damage, toxicity, weed extracts.

Introduction

The pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is a serious pest of mung bean and many other pulse grains in the tropics (Roy *et al.*, 2012a). The larvae of this pest penetrate into the pulse grains and feed endosperms, thus lead to damage grains as well as deteriorate nutritional value and germination capacity (Roy *et al.*, 2014). Different microorganisms, especially fungi develop in the infested grains and eventually make it unfit for human consumption and propagation (Deeba *et al.*, 2006).

Protection of pulse grains in the storage from the attack of *C. chinensis* mostly relied on synthetic insecticides like fumigation with methyl bromide and

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phosphine. These chemicals undoubtedly protect the grains but their excessive and inadvertent use created serious health hazards, environment pollution, cause ozone depletion and resistance to insects (Kim *et al.*, 2003). These problems demand the need for restriction of such chemicals to ensure pesticide free foodstuffs (Daglish, 2008). Therefore, environmentally safe and convenient methods such as the use of plant extracts, oils, leaf powders and pressurized carbon dioxide and temperature management techniques are the growing interest to replace synthetic pesticides (Yuya *et al.*, 2009).

Insecticidal activities of the plants have been intensively investigated and demonstrated promising for control of field and stored grain pests. Plant derived chemicals are hazard free and possess bitter substances which may show toxic, repellent, antifeedant, and growth and progeny inhibition activity against insect pests (Roy *et al.*, 2005; Roy *et al.*, 2014). Many plant-derived materials have been proved as toxic and growth regulators against stored products insects (Cosimi *et al.*, 2009). Plant lectins are biodegradable insecticidal agents that possessed deleterious effects on the survival, growth, oviposition and reproduction of stored grain insect pests (Oliveira *et al.*, 2011). The leaf extracts of ghagra *Xanthium strumerium* revealed insecticidal effectiveness against *C. chinensis* reared on black gram grains.

Plant powders, extracts and oils are a rich source of bioactive chemicals which reveal toxic effect and produce odors that repel adult beetles. The weed plants are excellent store of medicine and possess toxic chemicals, but little investigations have been done on their use in insect pest management. This study was designed with n-hexane extracts of five indigenous weed species to evaluate their effectiveness on mortality, fecundity and adult emergence inhibition of *C. chinensis* reared on mung bean grains. In addition, an assessment of the grain damage inhibition with the extracts was also determined.

Materials and Method

Insect culture

Mass culture of the insect was done on mung bean (*Vigna radiata*; Leguminosae) grains at an ambient temperature of 27 ± 2 °C and 80 ± 5 % RH in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. Ten pairs of adult beetles (1-3 day old) along with the food were placed in 11 glass jar. The mouth of the jar was fastened with muslin cloth with rubber band and kept 7 days in the laboratory for mating and oviposition of the insects. The beetles were removed from the jar and the eggs laid on the pulses were allowed to hatch. To study bioassay, insect rearing was repeated until 3rd generations with a view to ensuring enough population without affecting original culture.

Collection and preparation of weed sample

The weed species 'bhatpata', *Clerodendrum viscosum* Vent. (Verbenaceae), 'kashiature', *Cassia tora* L. (Leguminosae), 'dhakishak', *Dryopteris filix-max* L. (Polypodiaceae), 'bonmorich', *Croton bonplandianum* Baill. (Euphorbiaceae) and 'ghagra', *Xanthium strumarium* L. (Asteraceae) were collected from the road side of HSTU, collected in plastic bags and transported to the Entomology Laboratory. The weeds were washed with tap water and air dried for 7 days in the shade. Furthermore, the weeds were dried in an oven at 50 - 60°C for 24h to obtain constant weight. The weeds were powdered mechanically by using an electric blender (Braun Multiquick Immersion Hand Blender, B White Mixer MR 5550 CA, Germany), passed through 40 mesh screen and stored at 28 °C in tightly-closed dark glass bottles.

Preparation of n-hexane extracts from the weeds

Dried powder of each weed species was separately extracted in n-hexane. For each preparation, 10g powder was macerated in a 2.5 l capacity glass bottle using 1l n-hexane (96% analytical pure) for 7 days. To be sure for complete extraction, the sample was shaken for 72h using an electric shaker. The extract was filtered and the filtrate was considered as 1% concentration (1 g/100 ml). Similarly, 2 and 4% extracts were prepared and stored in a refrigerator at 4 °C until bioassay.

Contact toxicity test

Three day-old adult beetles were chilled for a period of 10 minutes in a refrigerator at 5 °C, and then 1µl of an extract was applied to the dorsal thorax of the beetles with a micro pipette. Fifty insects were used for each treatment and untreated control treatment was applied with n-hexane only. The insects were then transferred into 9 cm diameter Petri dishes (10 insects / Petri dish) that contained 100 g mung bean grains. Number of insect mortality in each Petri dish was recorded at 24, 48 and 72h after treatment and % mortality was calculated. Data of toxicity studies were corrected for untreated control mortality according to Schneider-Orelli's (1947) formula as mortalities in the control treatment ranged between 5 and 20%.

$$\text{Corrected mortality (\%)} = \frac{\% \text{ Mortality in treatments} - \% \text{ Mortality in control}}{100 - \% \text{ Mortality in control}} \times 100$$

Toxicity ratios (TR) were calculated using the formula: TR = LC₅₀ and /or LC₉₅ of the extract with less toxicity / LC₅₀ and /or LC₉₅ of the other extract, individually (Gusmao *et al.*, 2013).

Fecundity inhibition test

In each glass container 100 g grains were put and the predetermined extract concentrations were distinctly added to the containers with pipette, and subjected

to manual agitation for 2 min. Thereafter, the grains were placed in Petri dishes and five pairs of newly emerged beetles were released in each Petri dish. After 7 days, the numbers of eggs laid by the females on mung bean grains in the Petri dishes were counted by using hand lens. There were three replications for each extract concentration and a control treatment was made with untreated grains. The fecundity inhibition (%FI) of each extract concentration was calculated using the following formula:

$$\% \text{ FI} = \frac{\text{Eggs laid in control grains} - \text{Eggs laid in treated grains}}{\text{Eggs laid in control grains}} \times 100$$

Observation on adult insect emergence and grain damage inhibition

The weed extracts were poured distinctly into the glass containers which had mung bean grains. The extracts were mixed with the grains and then air dried. The grains were placed in different Petri dishes and five pairs of newly emerged beetles were released in each Petri dish. After 7 days the beetles were removed from the Petri dishes and the Petri dishes along with pulse grains were kept in the laboratory. Daily observation was made after one month of egg laid because bruchids adult usually emerge after 30 days of egg-laid. Observation was continued up to 15 days and removed the newly emerged beetles. A control treatment consisting of untreated grains was taken into account and each treatment replicated thrice. The adult emergence inhibition rate (%IR) was calculated by using the following formula:

$$\% \text{ IR} = \frac{\text{No. of insects in control grains} - \text{No. of insects in treated grains}}{\text{No. of insects in control grains}} \times 100$$

Number of damaged grains in each Petri dish was counted and percent grain damage inhibition (%DI) was calculated by the following formula:

$$\% \text{ DI} = \frac{\text{No. of damaged grains in control} - \text{No. of damaged grains in treatments}}{\text{No. of damaged grains in control}} \times 100$$

Statistical analysis

Probit analysis was employed in analyzing the dose-mortality response. LC_{50} and LC_{95} values and their fiducial limits were estimated. Data of the fecundity, adult emergence and grain damage inhibition were expressed as mean \pm SD (Standard Deviation). Significance of mean differences among the treatments were statistically compared using GLM at 5% probability level. The individual pair wise comparisons were made using Tukey's HSD posthoc analysis through SPSS (IBM SPSS statistics 21).

Results and Discussion

The extracts of the weeds at 24h after treatment showed toxicity effect on adult *C. chinensis* (Table 1). Contact toxicity data revealed LC₅₀ and LC₉₅ values from 5.3 (4.2-9.3) to 7.8 (5.3-23.4) and 10.2 (7.3-20.2) to 16.9 (10.6-57.7) g /100 ml, respectively. Results demonstrated that the χ^2 values of the data differed significantly ($p < 0.05$) and 'ghagra' extract was found to be the most effective. Its concentration response curve showed the steepest slope which indicated that small variations in the concentrations induced greater responses in mortality. The order of mortality activity of the weed extracts at 24h post treatment showed 'ghagra' > 'bonmorich' > 'dhakishak' > 'kashiature' > 'bhatpata'.

Table 1. Toxicity effect of five weed extracts on adult *Callosobruchus chinensis* exposed to 24 h post treatment

Weed plant	Slope (\pm S.E)	LC ^a 50 (95% fl)	TR ₅₀	LC95 ^a (95%fl)	TR ₉₅	χ^2 (df)
Bhatpata	0.20 \pm 0.05	7.8 (5.3 - 23.4)	-	16.9 (10.6 -57.7)	-	28.8 (13)
Kashiature	0.22 \pm 0.05	6.5 (4.4- 41.2)	1.20	15.3 (9.1-118.4)	1.10	36.8 (13)
Dhakishak	0.28 \pm 0.05	5.9 (4.4 - 11.6)	1.32	12.2 (8.4-27.4)	1.39	28.8 (13)
Bonmorich	0.31 \pm 0.05	5.3 (4.2 - 9.7)	1.47	11.0 (7.7-23.0)	1.54	34.0 (13)
Ghagra	0.33 \pm 0.05	5.3 (4.2 - 9.3)	1.47	10.2 (7.3-20.2)	1.67	36.6 (13)

Each datum represents the mean of five replicates, each set up with 10 adults (n = 50). Concentrations are expressed as g/ ml. fl stands for fiducial limits. ^aDifferent concentrations (1, 2 and 4g/100 ml). SE= Standards Error.

Table 2. Toxicity effect of five weed extracts on adult *Callosobruchus chinensis* exposed to 48 h post treatment

Weed plant	Slope (\pm S.E)	LC ^a 50 (95% fl)	TR ₅₀	LC95 ^a (95%fl)	TR ₉₅	χ^2 (df)
Bhatpata	0.13 \pm 0.04	6.5 (4.7 - 13.9)	-	18.7 (12.3 -46.2)	-	12.9 (13)
Kashiature	0.19 \pm 0.04	6.1 (4.4- 16.5)	1.07	14.9 (9.5-47.7)	1.26	25.4 (13)
Dhakishak	0.26 \pm 0.05	5.6 (4.6- 7.6)	1.16	11.9 (9.3-17.6)	1.57	13.9 (13)
Bonmorich	0.29 \pm 0.05	4.9 (3.9 - 8.5)	1.33	10.8 (7.7-21.9)	1.73	31.5 (13)
Ghagra	0.33 \pm 0.05	4.7 (4.1- 5.7)	1.38	9.6(7.9-12.5)	1.95	17.3 (13)

Each datum represents the mean of five replicates, each set up with 10 adults (n = 50). Concentrations are expressed as g/ ml. fl stands for fiducial limits. ^aDifferent concentrations (1, 2 and 4g/100 ml). SE= Standards Error.

The toxicity of the weed extracts against pulse beetle at 48h post treatment showed LC₅₀ and LC₉₅ values from 4.7 (4.1-5.7) to 6.5 (4.7-13.9) and 9.6 (7.9-12.5) to 18.7 (12.3-46.2) g/100 ml, respectively (Table 2). The results indicated that the χ^2 values of the data were significantly different ($p < 0.05$). The 'ghagra' extract revealed the lowest LC₅₀ and LC₉₅ values and the concentration response

curve of this plant also showed the steepest slope. The order of toxicity of the weeds at 48h post treatment was ‘ghagra’ > ‘bonmorich’ > ‘dhakishak’ > ‘kashiature’ > ‘bhatpata’.

When the n-hexane extracts of the weeds were examined for toxicity against *C. chinensis* at 72h post treatment, significant differences ($p < 0.05$) were observed (Table 3). The insecticidal activities of the weeds showed that their LC_{50} and LC_{95} ranged from 4.1 (3.4-5.7) to 6.0 (4.4-12.7) and 9.0 (6.9-14.3) to 18.6 (12.2-46.5) g/100 ml, respectively. Among the treatments, ghagra revealed the most toxic effect as it showed the lowest LC_{50} and LC_{95} values as well as steepest slope of the concentration curve. The order of toxicity of the weeds was ‘ghagra’ > ‘bonmorich’ > ‘dhakishak’ > ‘kashiature’ > ‘bhatpata’.

Table 3. Toxicity effect of five weed extracts on adult *Callosobruchus chinensis* exposed to 72 h post treatment

Weed plant	Slope (\pm S.E)	LC^{a50} (95% fl)	TR ₅₀	LC_{95}^a (95% fl)	TR ₉₅	χ^2 (df)
Bhatpata	0.14 \pm 0.04	6.0 (4.4 - 12.7)	-	18.6 (12.2 -46.5)	-	13.5(13)
Kashiature	0.25 \pm 0.04	4.6 (3.6- 8.3)	1.30	11.3 (7.8-24.5)	1.65	31.2 (13)
Dhakishak	0.28 \pm 0.04	4.6 (3.7- 7.2)	1.30	11.1 (8.1-19.8)	1.68	22.2 (13)
Bonmorich	0.31 \pm 0.04	4.2 (3.4-7.1)	1.43	10.0 (7.1-20.7)	1.86	38.1 (13)
Ghagra	0.36 \pm 0.05	4.1 (3.4 - 5.7)	1.46	9.0 (6.9-14.3)	2.07	28.9 (13)

Each datum represents the mean of five replicates, each set up with 10 adults ($n = 50$). Concentrations are expressed as g/ ml. fl stands for fiducial limits. ^aDifferent concentrations (1, 2 and 4g/100 ml). SE= Standards Error.

The toxicity of the extracts clearly showed that insect mortality varied with weed species, extract concentrations and exposure periods. The weed species acted as a valuable source of insecticide. The ingredients of these weeds may have the ability to inject into the body of the beetles and dysfunction their nutritional balance, thus caused mortality. Adedire and Akinneye (2004) reported the mortality effect of *Tithonia diversifolia* flower extracts on *C. maculatus*. Botanical insecticides offer broad spectrum toxic substances that interrupt insect's normal physiology and behavior, and influence on their feeding, mating, oviposition and mortality (Fouad *et al.*, 2014).

The fecundity inhibition effects of the weed extracts on *C. chinensis* are presented in Table 4. The weed species ($F_{4,30} = 8.9$, $p < 0.001$), extract concentrations ($F_{2,30} = 91.3$, $p < 0.001$) and interaction of weed species and extract concentrations ($F_{8,30} = 2.6$, $p < 0.05$) had significant effects on the fecundity. The extracts showed 31.7 ± 8.3 to $78.7 \pm 1.2\%$ fecundity inhibition and the most promising result was obtained by 4% ‘ghagra’. It may be that the weeds have compounds with broad spectrum action affecting the life stages of

the insect and inhibit their oviposition. Ambrósio *et al.* (2008) reported that the plant Mexican tounesol *Tithonia diversifolia* possessed sesquiterpene lactones which inhibited the oviposition of herbivore insects. The fecundity inhibition effects of the stem and flower extracts of kair *Capparis decidua* on caper *C. chinensis* have been reported by Upadhyay *et al.* (2006).

Table 4. Effect of five weed extracts on the fecundity inhibition (%mean \pm SD) of *Callosobruchus chinensis*

Weed plant	% Fecundity inhibition at different extract concentration.		
	1g/100 ml	2g/100 ml	4g/100 ml
Bhatpata	31.7 \pm 8.3bB	60.6 \pm 2.8aAB	66.6 \pm 7.1aA
Kashiature	48.1 \pm 5.7cA	57.6 \pm 6.9abB	68.1 \pm 1.3aA
Dhakishak	49.1 \pm 6.3bA	60.9 \pm 2.0abAB	66.2 \pm 6.8aA
Bonmorich	49.3 \pm 1.5bA	65.3 \pm 2.0aAB	68.3 \pm 6.1aA
Ghagra	52.7 \pm 3.2cA	67.5 \pm 1.5bA	78.7 \pm 1.2aA

Values followed by the same small letter(s) on the same row or by the same capital letter(s) in the same column are not significantly different, as assessed by Tukey HSD Posthoc ($p \leq 0.05$). SE= Standard Error.

Table 5. Effect of five weed extracts on the adult emergence inhibition (%mean \pm SD) of *Callosobruchus chinensis*

Weed plant	% Adult beetle emergence inhibition at different extract concentration.		
	1g/100 ml	2g/100 ml	4g/100 ml
Bhatpata	33.8 \pm 4.4cD	48.2 \pm 3.5abC	63.5 \pm 2.5aC
Kashiture	35.8 \pm 4.9cCD	54.6 \pm 2.0abC	73.8 \pm 1.5aB
Dhakishak	48.9 \pm 4.8bBC	64.5 \pm 5.4aB	66.5 \pm 3.4aC
Bonmorich	53.0 \pm 5.8bB	72.5 \pm 3.4aAB	78.2 \pm 2.4aAB
Ghagra	76.9 \pm 5.1cA	78.2 \pm 2.4bA	81.1 \pm 1.4aA

Values followed by the same small letter(s) on the same row or by the same capital letter(s) in the same column are not significantly different, as assessed by Tukey HSD Posthoc ($p \leq 0.05$). SE= Standard Error.

The weed species ($F_{4,30} = 86.7$, $p < 0.001$), extract concentrations ($F_{2,30} = 139.2$, $p < 0.001$) and interaction of weed species and extract concentrations ($F_{8,30} = 9.7$, $p < 0.001$) showed significant effect on the adult beetle emergence (Table 5). Adult beetle emergence inhibition in different treatments varied from 33.8 \pm 4.4 to 81.1 \pm 1.4%. Among the treatments, 4% ghagra and 1% bhatpata revealed the highest and lowest level of inhibition, respectively.

Nennah (2011) reared *T. castaneum* and *R. dominica* on ground wheat grains mixed with methanolic extracts of harmel *Peganum harmala* seed and found abnormal larvae and pupae, as well as dose dependent adult emergence inhibition of the insects. In the present study, the weed extracts showed remarkable effect

on the adult emergence of *C. chinensis*. The extracts revealed 33.8 to 81.1% adult emergence inhibition and the inhibitory activity of the extracts increased with increased concentration. Roy *et al.* (2012b) observed 37.0% adult emergence inhibition of *C. chinensis* with 4% aqueous extract of common cocklebur, *X. strumarium*.

Table 6 showed that the extracts possessed grain damage inhibition against *C. chinensis*. The effects of weed species ($F_{4,30} = 22.8$, $p < 0.001$), extract concentrations ($F_{2,30} = 92.6$, $p < 0.001$) and interaction of weed species and extract concentrations ($F_{8,30} = 3.9$, $p < 0.01$) differed significantly. Grain damage inhibition among the treatments varied from 10.3 ± 7.2 to $60.1 \pm 3.8\%$, and the highest and lowest inhibition levels were attained by 4% ghagra and 1% bhatpata, respectively. The present findings indicated that the weed extracts had potential effect to control stored grain pest. The toxicity of the extracts might hamper food ingestion and feeding activity of the pests. Tavares *et al.* (2011) observed that the extracts of tounesol *T. diversifolia* reduced egg hatching of *Spodoptera frugiperda* and inhibited grain damage of *Triticum aestivum* both in the field and storage conditions.

Table 6. Effect of five weed extract on the grain damage inhibition (%mean \pm SD) of mung bean from the attack of *Callosobruchus chinensis*

Weed plant	% Grain damage inhibition at different extract concentration.		
	1g/100 ml	2g/100 ml	4g/100 ml
Bhatpata	10.3 ± 7.2 cB	26.8 ± 2.7 bBC	50.2 ± 3.1 aA
Kashiture	18.9 ± 7.3 bAB	38.7 ± 5.7 aB	39.6 ± 3.6 aB
Dhakishak	20.6 ± 6.8 cAB	24.4 ± 6.5 abC	38.6 ± 5.5 aB
Bonmorich	23.3 ± 6.3 bAB	52.2 ± 5.5 aA	59.8 ± 3.9 aA
Ghagra	28.6 ± 2.8 bA	57.0 ± 1.3 aA	60.1 ± 3.8 aA

Values followed by the same small letter(s) on the same row or by the same capital letter(s) in the same column are not significantly different, as assessed by Tukey HSD Posthoc ($p \leq 0.05$). SE= Standard Error.

Grain protection from the attack of insect pests without hampering environment is the main objective of the application of botanical pesticides. Plant materials possessed antifeedant substances and deterred insects from feeding and damaging grains (Amin *et al.*, 2000; Shahjahan and Amin, 2000; Roy *et al.*, 2010). The studied weed extracts protected mung bean grains from the attack of *C. chinensis* to a significant level (10.3 to 60.1%). This finding indicated that the weed extracts inhibited feeding behavior of the pest and protected the grains. Other authors have obtained similar results with different plant and insect species. Rahman and Talukder (2006) reported that 3% acetone extract of lagundi *Vitex negundo* revealed good protection for black gram seeds against *C. chinensis* infestation.

Plant materials are easy to manufacture and application and the studied weed species are very common in the rural areas of Bangladesh. The mixtures of weed materials with rapid and slow action insecticide might be useful in the protection of stored grains. In the present study, 4% 'ghagra' extract demonstrated the highest mortality and inhibited fecundity, adult emergence, as well as protected grains from infestation. So, the findings suggested that the 'ghagra' leaf extract as an alternative means of chemical insecticides may be used to save mung bean grains from the attack of *C. chinensis*. Further studies are needed to identify the toxic compounds, cost of treatment, effect of the odor and flavor on processed grains, toxicity of extract on non-target species including human and development of formulation for effective application.

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